MICROSPORES AND POLLEN GRAIN IN TRIPLOID *CHONDRILLA JUNCEA* L. FROM UNPOLLUTED AND POLLUTED AREAS

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Preliminary observations of plants collected at a natural locality in Jany (Zielona Góra district, Poland) suggested that in some plants dyads occurred mixed with more or less regular tetrads, monads, triads and polyads. Similar results were obtained in plants growing on an unpolluted site at an experimental field in Modlnica near Cracow and in a highly polluted area close to a postfloatation reservoir at the Żelazny Most copper mine near Rudna (Silesia). However, in the plants growing in contaminated soil a higher degree of degeneration processes was observed. Either dyads or tetrads prevailed in the capitula in the analyzed plants. In some of their loculi, dyads and tetrads were mixed with monads, dyads, triads and/or polyads. Microcytes and pollen grains of different sizes were common. The sterility of mature pollen grain was slightly higher in a plant from Żelazny Most (80–85%) than in its derivative from Modlnica (65–75%). Degeneration of whole anthers in the plant from the polluted locality was frequent. In some anthers the destruction of meiocytes started early, together with precocious abortion of the anther tapetum.

Key words: *Chondrilla juncea*, disturbances of pollen grain formation, polluted and unpolluted conditions.

INTRODUCTION

The data concerning microsporogenesis in *Chondrilla juncea* L., an autonomous apomict with a triploid chromosome number (2n = 15), differ in details, and suggest the possibility of some genetic variations among populations. Rosenberg (1912) was the first to investigate the mode of reproduction of *C. juncea,* stating that the pollen mother cells (PMCs) divided only once, so that dyads were formed exclusively during microsporogenesis. Later, Poddubnaya-Arnoldi (1933) characterized the formation of dyads, triads, pentads and hexads in that species. Bergman (1950) described two clones of *C. juncea* from the Swedish Botanic Gardens which differed from each other in their pollen formation. Normal tetrads were formed in a clone from Uppsala. Microsporogenesis in plants from Stockholm nearly always produced dyads. A preliminary examination of plants collected from a polluted site in the close vicinity of a copper mine postfloatation reservoir and from an uncontaminated experimental field suggested that in some plants dyads occurred mixed with more or less regular tetrads, monads, triads and polyads. Previous observations of *C. juncea* from natural habitats in Poland (Kościńska-Pająk, 1996) showed differences in pollen grain size and high pollen sterility.

The present study was focused on meiosis II and the formation of microspores and pollen grains in plants from natural habitats or cultivated on an experimental field. The observed abnormalities in the processes were connected chiefly with disturbances usually described in cytologically unbalanced triploids. Attention was also paid to the possible influence of a polluted environment on these processes. Problems concerning plants growing in heavily polluted conditions have been covered

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in several comprehensive reviews, but the authors were mainly concerned with plant tolerance of heavy metals and its mechanisms (Gregory and Bradshaw, 1965; Cox and Hutchinson, 1979, 1980; Symeonidis et al., 1985; Karataglis, 1986; Baker, 1987; Antosiewicz, 1992; Ernst et al., 1992; Woźny and Krzesłowska, 1993; Wierzbicka and Panufnik, 1998) or with their accumulation in plants (Godzik, 1993; Mesjasz-Przybyłowicz et al., 1998, 1999). The toxic effects of heavy metals on some life processes in plants, for example seed development and germination, have been covered in some studies (Lane and Martin, 1977; Woźny et al., 1982).

The number of studies on the influence of heavy metals on embryological processes is relatively small (Searcy and Mulcahy, 1985a,b; Izmaiłow, 1999). It may be well to add that some disturbances of pollen formation have been observed in trees exposed to traffic emissions (e.g., Ostrolucká, 1989; Ostrolucká et al., 1995).

This study investigated plants growing at the base of a reservoir with postfloatation wastes from copper ore processing. The most significant pollution factors in the neighborhood of the reservoir are high wind erosion of the reservoir and its slopes, and infiltration of water from the reservoir and from polluted groundwater into the soil. The water contains heavy metals (Cu, Pb, Mn), ions (Cl^-, SO_4^{2-}, Na^+, Ca^{2+}) and floatation substances (Czaban and Maślanka, 1998; Kijewski, 1998). This area, particularly the slopes, were relatively poorly colonized by plants.

This paper compares the stages of meiosis II in three plants: one from a natural unpolluted locality, the second growing at a polluted site, and the third derived from seeds of the latter plant but cultivated from the beginning in unpolluted conditions.

MATERIALS AND METHODS

Three plants of *C. juncea* were chosen. One was growing at a natural locality in Jany (Zielona Góra district, Poland), the second at the base of the Żelazny Most copper mine waste postfloatation reservoir near Rudna (Legnica-Głogów Copper Belt, Silesia, Poland). The third plant grew in uncontaminated soil at an experimental field in Modlnica near Cracow. The latter plant derived from seeds collected from the investigated specimen from Żelazny Most. Jany and Modlnica are situated in areas where contamination of the environment does not exceed Polish environmental norms. Neither pesticides nor chemical fertilizers were used on the experimental field.

Ten capitula approximately the same age were taken from each plant just before anthesis and fixed in ethanol/acetic acid (3:1). Paraffin-embedded material was cut to 10 μm and stained in Heidenhain's hematoxylin with alcian blue. Three samples of shedding pollen grains taken from the heads of each plant were tested with acetocarmine to determine the percentage of stained (with cytoplasm and nuclei) and unstained (empty) pollen grains.

RESULTS

PLANTS FROM JANY AND MODLNICA

In general the results of examining II meiotic division material from the plant growing in Jany and on the experimental field in Modlnica were the same. Detailed examination of 10 capitula taken from different parts of the plant cultivated in Modlnica showed that mainly dyads were formed in 3 of the 10 capitula. They contained regular nuclei of equal size (Fig. 1), or sometimes one nucleus was smaller. In the loculi with dyads, single monads, triads or tetrads occurred. In some triads the cells showed distinct size differentiation (Fig. 3). The micronuclei observed in such triads originated from lagging chromosomes which were observed in some II anaphases. In 7 capitula mainly tetrads were observed, together with a small number of other forms. Usually the tetrads were normal, isobilateral, with nuclei more or less of equal size (Fig. 2a,b). In some tetrads the cells showed distinct size differentiation (Fig. 4). In other loculi, monads, dyads, pentads and hexads were observed together with regular and disturbed tetrads. The presence of different numbers and sizes of cells and nuclei was probably the result of disturbances in the second meiotic division in which lagging chromosomes were observed between one or both anaphase groups.

In both plants the anthers did not show any evidence of precocious degeneration of the tapetal cells; their destruction began at the moment of separation of the microspores. Totally aborted dyads or tetrads were not observed.

For the plant from Modlnica, the acetocarmine test showed 25% stained pollen grains in the first sample, and 30% and 35% in the other two. For the plant from Jany the percentages of stained pollen grains were 26%, 29% and 36%. In both plants the diameters of stained pollen grain ranged from 37.5 μm to 67.5 μm, and from 7.5 μm to 45 μm for unstained ones (Fig. 11a,b).

PLANT FROM ŻELAZNY MOST

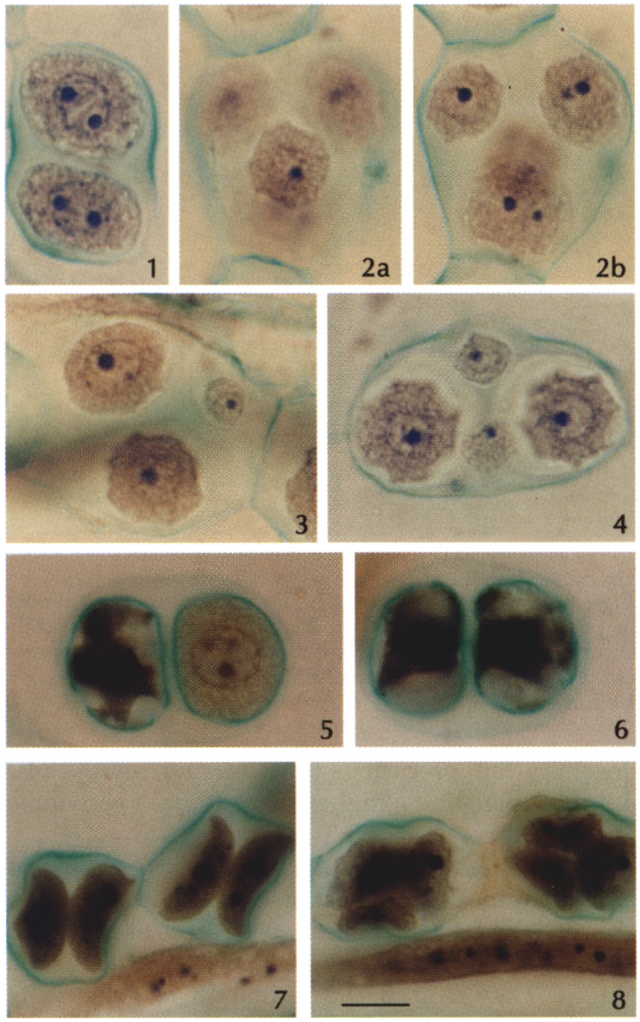
The meiosis II and pollen formation results from the above plants were compared with those from the plant growing in polluted soil at Żelazny Most. The results on formation of microspores were comparable with those for the plants from Jany and Modlnica. In 4 capitula, dyads prevailed among a small number of other possible microspore configurations. In the other 6, tetrads were mainly formed, apart from single cases of other possible configurations. Monads, dyads, triads, pentads and hexads were more numerous in some florets.

Degenerative processes took place in all capitula. In many loculi in which mainly dyads were formed a large number of them aborted (Figs. 6, 7). The microspore walls collapsed, and their nuclei became deformed. Sometimes only one cell of the dyad degenerated, whereas the second one was viable (Fig. 5). There were loculi with all aborted dyads, and others filled with viable dyads. Totally aborted anthers containing tetrads were also observed. The nuclei in cells of degenerated tetrads were deformed and surrounded by dense, dark-stained cytoplasm (Fig. 8).

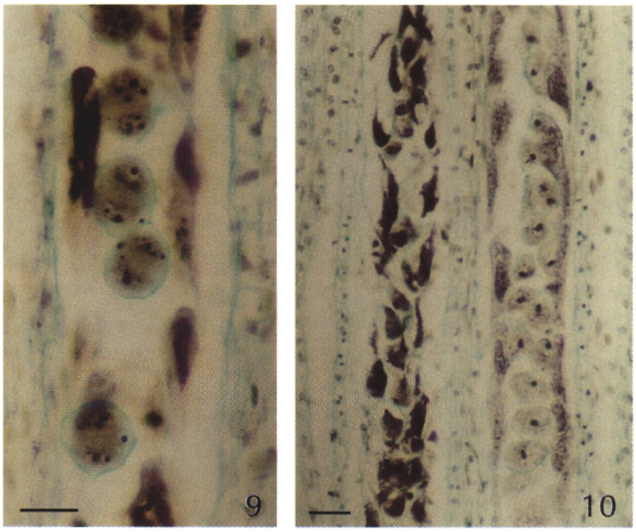
In *C. juncea,* apoptosis of tapetal cells starts at the moment of separation of the microspores. Thus, in the majority of loculi in which degeneration of dyads or tetrads was observed the tapetal cells showed the first evidence of degeneration. In the same stage, single cells of tapetum were aborted. In some anthers the processes of degeneration might start very early, beginning from the tapetum: aborted tapetal cells were observed at prophase I. Sometimes in such cases there were still viable PMCs present (Fig. 9), but simultaneous total abortion of PMCs and tapetal cells was usually observed (Fig. 10).

Examination of three samples of shedding pollen grains collected from the plant from Żelazny Most showed a high level of pollen sterility. The percentage of stained pollen grains in the acetocarmine test in the first sample was 15%, in the second 16% and in the third 20%. Pollen grain diameter ranged from 30 μm to 60 μm for stained pollen grains and from 7.5 μm to 45 μm for unstained ones. The mean diameter of stained pollen grains was 44.32 μm, and 25.8 μm in unstained ones (Fig. 11c).

In triploid *C. juncea* with odd chromosome numbers the formation of dyads, tetrads and other configurations of microspores, the result of irregular meiotic division, was not correlated with individual plants or with external conditions. This result is in agreement with Poddubnaya-Arnoldi (1933), who interpreted the formation of different microspore configurations as the result of disturbed meiosis in plants with an odd triploid number of chromosomes. Bergman's (1950) results may point to genetically fixed disturbances of meiosis which lead to dyad formation. The possibility of such differentiation was not confirmed in our study. On the other hand, our results indicated the influence of the components of polluted soil and atmosphere on pollen grain formation, producing higher levels of microspore



Figs. 1–8. *Chondrilla juncea* L. Configurations of microspores in plant from Modlnica (Figs. 1–4) and Żelazny Most (Figs. 5–8). **Fig. 1.** Dyad with nuclei of equal size. **Figs. 2a–b.** Tetrad with nuclei of equal size (in two foci). **Fig. 3.** Triad with one small nucleus. **Fig. 4.** Tetrad with two small and two big nuclei. **Figs. 5–7.** Degenerating dyads. **Fig. 5.** With one viable nucleus. **Fig. 6.** With two degenerating nuclei. **Fig. 7.** With totally deformed nuclei. **Fig. 8.** Aborted, deformed tetrad. Bar in Fig. 8 = 10 μm and corresponds to all figures.



Figs. 9–10. *Chondrilla juncea* L. Degeneration of tapetal cells in plant from Żelazny Most. **Fig. 9.** Aborted tapetal cells observed at prophase I; note that still-viable PMCs are present. **Fig. 10.** Total abortion of PMCs and tapetal cells. Bar = 10 µm.

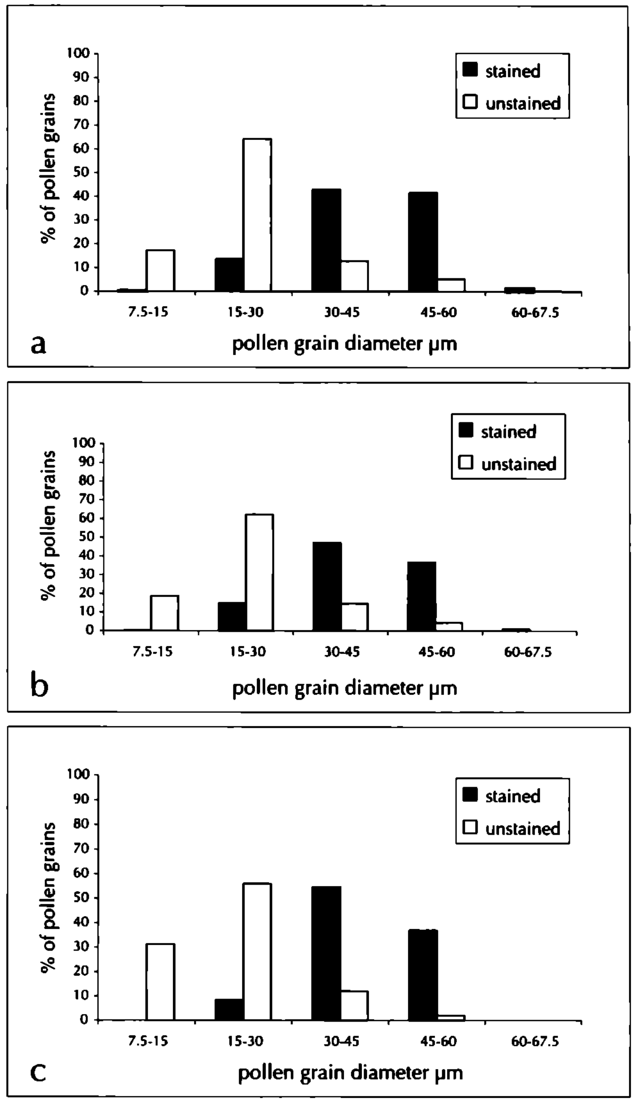


Fig. 11a–c. Differentiation of pollen grain diameter in plants of *Chondrilla juncea*. (**a**) From Jany, (**b**) From Modlnica, (**c**) From Żelazny Most.

degeneration, precocious tapetum and young PMC degeneration, and a higher percentage of nonviable pollen grains.

The differences in diameter between stained and unstained pollen grains as well as between groups may be interpreted as a result of irregular conjugation of chromosomes and the next meiotic stages. They caused the formation of genetically unbalanced pollen grains with various chromosome number combinations and types.

DISCUSSION

It is well known that metallurgical processing of copper, zinc and lead is responsible for considerable amounts of heavy metals introduced into the environment (Brej, 1998). Problems of sensitivity and tolerance to heavy metals in plants growing in heavily polluted conditions have been covered in several comprehensive reviews by Baker (1987), Antosiewicz (1992), Ernst et al. (1992) and others. However, data on the influence of heavy metals for embryological processes are rather scarce and sometimes suggest high tolerance to pollution. For example, Searcy and Mulcahy (1985) stated that in *Silene dioica* an increased amount of copper in flowers had no effect on pollen quality in plants homozygous for copper tolerance. However, the increases of copper and zinc in the flowers were accompanied by a reduction of the percentage of viable pollen in both heterozygous clones. On the other hand, there are data suggesting that male reproductive organs are sensitive to the influence of traffic emissions (Ostrolucká, 1989; Ostrolucká et al., 1995). The higher degree of degeneration observed in *C. juncea* from the polluted area is interpreted to be caused by heavy metals and other chemicals originating from the copper extraction processes which have passed to the soil from groundwater and from the air. In the case of *C. juncea*, degeneration of the microspores, tapetal cells and whole anther was connected with the influence of heavy metals, which probably accumulate in inflorescences, particularly since such degeneration had not been observed in material collected in Modlnica. The observations of plants of *Vicia cracca* growing at Żelazny Most also showed a wide range of size differences of mature pollen grains, and their almost complete degeneration in some of the anthers (Izmaiłow, 1999).

However, *C. juncea* does grow in that polluted environment and is able to reproduce there; seedlings and young plants were found around the old plants. This evidences the tolerance of *C. juncea* to substances in the soil and air at that locality.

According to Tomsett and Thurman (1988) the following types of tolerance are recognized: (1) constitutional tolerance, which refers to plants growing in unpolluted areas; such plants have never been in contact with a given metal but nevertheless exhibit high tolerance to a potentially toxic level of this metal; (2) co-tolerance, meaning tolerance developed towards one metal which confers tolerance to a different metal or metals with which the plant was not exposed to previously; and (3) multiple tolerance, said of a population of plants having tolerance to two or more metals whose levels in the soil have become elevated. Probably *C. juncea* belongs to the latter group of plants. The soil contained such heavy metals as Cu, Pb and Mn, the ions Cl^-, SO_4^{2-}, Na^+ and Ca^{2-}, and postfloatation substances, but the plant is able to reproduce in this habitat.

The results suggest that the population of *C. juncea* growing at the contaminated site is to some degree genetically tolerant to the specific polluted conditions of that environment.

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